## REMARKS

Claims 1 to 12 are pending in the application. Claims 1-3 and 6-11 have been withdrawn from consideration, as drawn to a non-elected invention. Per this amendment, claims 4-5 and 12 have been amended. Claims 4-5 and 12 are currently under consideration.

As a preliminary matter, Applicants would like to offer some comments regarding the screening methods currently claimed. Claim 4 recites a "method of screening for a substance that inhibits acylated homoserine lactone." To put the claim in context, among the uses of the inhibitor is "as a biofilm inhibitor and as filter- and pipe-clogging inhibitors." In addition, because such inhibitors "inhibit the expression of pathogenic factors in microorganisms, it can also be used as a therapeutic agent against infectious disease." *See* [0117]. *See also* [0012]-[0014]. Nonetheless, the screening method does not involve the assessment of biofilm build-up nor the expression of pathogenic factors. Instead, the screening method relies on the ability of acylated homoserine lactone to inhibit the activity of Akt in animal cells; more specifically, the method relies on the ability of an inhibitor of acylated homoserine lactone to inhibit that inhibition of Akt.

Element (ii) of claim 4 has been amended to more precisely reflect the detection of that inhibition. Specifically, element (ii) has been amended to recite ". . . detecting one or more of

(a) phosphorylated-Akt,

> wherein increased phosphorylation reflects inhibition of acylated homoserine lactone,

(b) apoptosis, or (c) caspase activity,

> wherein the apoptosis or caspase activity is modulated by Akt and
>
> wherein decreased apoptosis or decreased caspase activity reflects inhibition of acylated homoserine lactone . . . ."

Support for this amendment can be found in the specification, for example, at least at paragraphs [0072], [0075], [0093]-[0095], [0097]-[0099] and at Figures 3 and 15-18, which disclose various methods for detecting Akt activity, apoptosis, and caspase activity. For example, paragraph [0072] states that "specific acylated homoserine lactone effectively inhibits the phosphorylation of Akt." According to paragraph [0094], Akt becomes activated via phosphorylation and "activated Akt inhibits apoptosis in cells." As set forth in paragraph [0095], "[s]ince Akt inhibits apoptosis via various substrates, inhibition of Akt activity has an effect on other molecules that are responsible for the survival signalling pathway in which Akt is involved, and finally causes apoptosis in cells." One such molecule is caspase. According to paragraph [0098] "[w]hen an inactive precursor of caspase is activated by limited degradation, a protease cascade is formed within the cells and apoptosis is carried out." Thus, "caspase activation refers to the inhibition of the survival signalling pathway in which Akt is involved," as also stated in paragraph [0098]. Accordingly amended claim 4 is fully supported by the application as filed. Amendments to claims 5 and 12 parallel the amendments made to claim 4 merely to promote claim consistency. Accordingly, those amendments are fully supported by the application as filed as well. No new matter is added by the amendments to the claims.

**Preliminary Matters**

Priority

Applicants thank the Examiner for acknowledging receipt of the papers submitted under 35 U.S.C. 119(a)-(d) and receipt of the translation of the JP 2003-21053 application.

Drawings

Applicants thank the Examiner for acknowledging receipt of the Petition to Accept Color Photographs under 37 C.F.R. § 1.84(b)(2) and respectfully await a decision from the appropriate persons.

**Claim Rejections under 35 U.S.C. § 112, second paragraph**

The Examiner rejected claim 4 under the second paragraph of 35 U.S.C. § 112 as allegedly "being incomplete for omitting essential elements." Office Action, at page 2. The Examiner alleged that claim 4 omitted the step of "how inhibition of Akt activity is being detected." *Id.* The Examiner stated that "the detection step could reasonably [be] interpreted as detecting Akt itself or alternatively, as detecting apoptosis which is an activity of Akt." *Id.* The Examiner indicated that "[f]or examination purposes, detecting inhibition of Akt activity will be interpreted as detecting apoptosis or detecting caspase activity." *Id.* at page 3.

Applicants respectfully traverse. Nonetheless, solely to facilitate prosecution, and not in acquiescence to the Examiner's rejection, Applicants have amended claim 4 to recite

". . . detecting one or more of

(a) phosphorylated-Akt,

> wherein increased phosphorylation reflects inhibition of acylated homoserine lactone,

(b) apoptosis, or (c) caspase activity,

> wherein the apoptosis or caspase activity is modulated by Akt and wherein decreased apoptosis or decreased caspase activity reflects inhibition of acylated homoserine lactone . . . ."

Applicants therefore respectfully request withdrawal of the rejection.

**Claim Rejections under 35 U.S.C. § 103**

Claims 4 and 5

The Examiner rejected claims 4-5 under 35 U.S.C. § 103(a) as allegedly "being unpatentable over Pearson et al. (US 5,591,872, 1997, IDS) in view of Smith et al. (J. Immunol. 2001: 167: 366-374, IDS), Koo et al. (US 2002/0054869, 2002) and Rajan et al. (Am. J. Respir. Cell Mol. Biol. 2000; 23: 304-312)." Action at page 3.

As in the Office Action of March 27, 2007, the Examiner alleged that Pearson teaches "a method of selecting inhibitors of the autoinducer molecule, N-(3oxododecanoyl) homoserine lactone, comprising contacting the autoinducer molecule with a suspected inhibitor, measuring the ability of the treated autoinducer molecule to stimulate the activity of a selected gene then determining whether the inhibitor represses or enhances the activity of the autoinducer molecule (column 5, lines 46-55)." *Id.* The Examiner stated that "[Pearson] further teaches a method of inhibiting the infectivity of *P. aeuriginosa* and methods of treating an immuno-compromised host infected by *P. aeruginosa,* e.g., a person afflicted with cystic fibrosis (column 6, lines 22-26)." *Id.* The Examiner, however, again recognized that Pearson "do[es] not explicitly teach that the method comprising culturing animal cells with the test agent and acylated homoserine lactone and detecting the inhibition of Akt by detecting apoptosis." *Id.*

The Examiner relied on Smith for allegedly teaching:

> [A] method of determining the affects of 3-O-C12- HSL (N-3oxododecanoyl homoserine lactone) on MAP kinases, comprising contacting 16HBE cells with a test substance such as an inhibitor of the MAP kinase signaling pathway in the presence of 3-O-C12-HSL and determining the activation of ERK (page 371, 1st column, 1st full paragraph to 2nd column). In particular, the reference teaches that 3-O-C12-HSL activates the MAP kinase signaling pathway which is important in IL-8 production (page 371, 1st column, 1st full paragraph).

*Id.* The Examiner further alleged that "[Smith] teaches that 3-O-C12-HSL also induces NF-kB and AP-2 which subsequently upregulates IL-8 which leads to neutrophil infiltration and inflammation found in *P. aeruginosa* infection (page 373, 2nd column, last paragraph)." *Id.* at pages 3-4.

The Examiner stated that Koo "teach[es] that inhibition of the MAP kinase signaling pathway specifically triggers an apoptotic response in human cells (paragraph 0010)." *Id.* at 4. The Examiner also stated that Koo "further teach[es] that inhibitors of the MAP kinase signaling pathway such as PD9805[9] are useful for inhibiting the growth of a tumor in a mammal, wherein the inhibitor induces a cytotoxic response leading to apoptosis of cells in said mammal (Claims 16-20 of US 2002/0054769)." *Id.*

The Examiner contended that Rajan "teach[es] the induction of apoptosis by *Pseudomonas aernginosa* in respiratory epithelial cells. In particular, the reference teaches that the resistance of airway epithelial cells to apoptosis is due to the stimulation of NF-κB by adherent *P. aernginosa,* wherein NF-κB appears to have an antiapoptotic effect in respiratory cells." *Id.*

Thus, the Examiner concluded that "[i]t would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to culture a test substance in the presence of N-3-oxodocecanoyl homoserine lactone as taught by Pearson et al. in an animal cell such as a neutrophil and to identify an inhibitor of N-3-oxodocecanoyl homoserine lactone by detecting apoptosis in view of the teachings of Smith et al., Koo et al. and Rajan et al." *Id.* For motivation, the Examiner pointed to Smith's alleged teaching that "3-O-C12-HSL induces MAP kinases, as well as NF-κB, each of which are known in the art to be involved in apoptosis in view of the teachings of Koo et al. and Rajan et al." *Id.* The Examiner finally contended that:

> [O]ne of ordinary skill in the art would have a reasonable expectation of success that by culturing a test substance in the presence of N-3-oxodocecanoyl homoserine lactone as taught by Pearson et al. in an animal cell such as a neutrophil and to identify an inhibitor of N-3-oxodocecanoyl homoserine lactone by detecting apoptosis in view of the teachings of Smith et al., Koo et al. and Rajan et al., one would achieve an effective method of identifying a suitable inhibitor for the treatment of an immunocompromised host infected by *P. aerrginosa,* e.g., a person afflicted with cystic fibrosis.

*Id.*

Applicants respectfully traverse. To support a rejection under 35 U.S.C. § 103, the Examiner must clearly articulate the reasons why the claimed invention would have been obvious. Such an analysis should be made explicit and cannot be premised upon mere conclusory statements. *See* MPEP § 2142, 8th Ed., Rev. 6 (Sept. 2007). Furthermore, "[t]he mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art" at the time the invention was made. MPEP § 2143.01(III) (internal citation omitted). Moreover,"[i]n determining the differences between the prior art and the claims, the question under 35 U.S.C. § 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious." MPEP § 2141.02(I) (internal citations omitted).

Here, the claimed invention as a whole is not obvious for at least the reason that the references, whether taken alone or in combination, fail to teach or suggest every element recited in the claims. Not one of the references teaches or suggests "[a] method of screening for a substance that inhibits acylated homoserine lactone, comprising . . . detecting one or more of (a) phosphorylated-Akt, wherein increased phosphorylation reflects inhibition of acylated

homoserine lactone, (b) apoptosis, or (c) caspase activity, wherein the apoptosis or caspase activity is modulated by Akt and wherein decreased apoptosis or decreased caspase activity reflects inhibition of acylated homoserine lactone . . . ." Indeed, the Examiner conceded that Pearson "do[es] not explicitly teach that the method comprising culturing animal cells with the test agent and acylated homoserine lactone and detecting the inhibition of Akt by detecting apoptosis." Action at page 3. This deficiency in Pearson is not remedied by Smith, Koo, or Rajan.

Previously, the Examiner conceded that Smith "do[es] not specifically teach that Erk is involved in the survival signaling pathway in which Akt is involved" in a rejection based on inherent anticipation. Office Action issued March 27, 2007, at page 5. In addition, the Examiner previously conceded that Smith "do[es] not explicitly teach that the inhibition of ERK is determined by detecting apoptosis." *Id.* at page 6. This rejection, however, was withdrawn in light of Applicant's arguments. *See* Action at page 6 and Response to Office Action mailed June 27, 2007. Now, the Examiner adds that Smith teaches that (1) "3-O-C12-HSL activates the MAP kinase signaling pathway which is important in IL-8 production (page 371, 1st column, 1st full paragraph)" and (2) "3-O-C12-HSL also induces NF-kB and AP-2 which subsequently upregulate[] IL-8 which leads to neutrophil infiltration and inflammation found in *P. aeruginosa* infection (page 373, 2nd column, last paragraph)." Action at pages 3-4. The Examiner, however, does not provide any reasoning as to how these alleged teachings of Smith relate to a method comprising "detecting one or more of (a) phosphorylated-Akt, wherein increased phosphorylation reflects inhibition of acylated homoserine lactone, (b) apoptosis, or (c) caspase activity, wherein the apoptosis or caspase activity is modulated by Akt and wherein decreased apoptosis or decreased caspase activity reflects inhibition of acylated homoserine lactone" as

recited in the instant claims. In fact, not one of the documents cited by the Examiner even suggests that Akt is involved in the observed induction of NF-κB or AP-2. Indeed, the Examiner has not provided any rationale for connecting inhibition of the Raf-MEK-ERK MAP kinase signaling pathway with "[a] method of screening for a substance the inhibits acylated homoserine lactone, comprising . . . detecting one or more of (a) phosphorylated-Akt, wherein increased phosphorylation reflects inhibition of acylated homoserine lactone, (b) apoptosis, or (c) caspase activity, wherein the apoptosis or caspase activity is modulated by Akt and wherein decreased apoptosis or decreased caspase activity reflects inhibition of acylated homoserine lactone . . . ."

Smith does not show anything regarding the Akt signaling pathway. Instead, Smith shows that a different signaling pathway, i.e., the Raf-MEK-ERK MAP kinase signaling pathway, is involved in the NF-κB induction. Specifically, Smith shows that "[t]he addition of a MEK inhibitor[, PD98095,] with 3-O-C12-HSL stimulation failed to induce nuclear mobilization of NF-κB," a necessary step in the transcription of NF-κB-responsive genes. Smith at page 372, right column. Smith also stated that "[w]hen 16HBE cells were cocultured with 3-O-C12-HSL and 50 μM of PD98059, the production of IL-8 protein was reduced by 80-90% (Fig. 8B)." *Id.* at page 371, right column. Indeed, Smith concluded that "[a]ctivation of IL-8 and NF-κB occurs through ERK activation." *Id.* at 372, legend for Figure 8. Thus, Smith does not teach a method comprising "detecting one or more of (a) phosphorylated-Akt, wherein increased phosphorylation reflects inhibition of acylated homoserine lactone, (b) apoptosis, or (c) caspase activity, wherein the apoptosis or caspase activity is modulated by Akt and wherein decreased apoptosis or decreased caspase activity reflects inhibition of acylated homoserine lactone"

because it shows that inhibition of a different pathway, i.e., the Raf-MEK-ERK MAP kinase signaling pathway, is sufficient to block NF-κB translocation to the nucleus and the vast majority of IL-8 production. Accordingly, activation of the Raf-MEK-ERK MAP kinase signaling pathway does not teach or suggest any involvement of the Akt signaling pathway.

As also set forth in the Response to Office Action mailed June 27, 2007, Koo teaches that inhibition of the Raf-MEK-ERK MAP kinase signaling pathway selectively induced apoptosis in melanoma cells. Again, nowhere does Koo mention the Akt pathway or acylated homoserine lactone. The Examiner has again not provided any rationale for connecting inhibition of the Raf-MEK-ERK MAP kinase signaling pathway with "[a] method of screening for a substance that inhibits acylated homoserine lactone, comprising . . . detecting one or more of (a) phosphorylated-Akt, wherein increased phosphorylation reflects inhibition of acylated homoserine lactone, (b) apoptosis, or (c) caspase activity, wherein the apoptosis or caspase activity is modulated by Akt and wherein decreased apoptosis or decreased caspase activity reflects inhibition of acylated homoserine lactone. . . ."

Regarding Rajan, the Examiner has not clearly articulated how NF-κB's "antiapoptotic effect in respiratory cells" is related to "detecting one or more of (a) phosphorylated-Akt, wherein increased phosphorylation reflects inhibition of acylated homoserine lactone, (b) apoptosis, or (c) caspase activity, wherein the apoptosis or caspase activity is modulated by Akt and wherein decreased apoptosis or decreased caspase activity reflects inhibition of acylated homoserine lactone." In fact, nowhere does Rajan mention or discuss the Akt pathway. As demonstrated by Smith above, NF-κB activation may be dependent on the Raf-MEK-ERK MAP kinase signaling pathway. Accordingly, NF-κB activation does not teach or suggest anything regarding the Akt signal transduction pathway.

Thus, Pearson, Smith, Koo, and Rajan, alone or in combination, do not render the instant claims obvious because they do not teach or suggest "detecting one or more of (a) phosphorylated-Akt, wherein increased phosphorylation reflects inhibition of acylated homoserine lactone, (b) apoptosis, or (c) caspase activity, wherein the apoptosis or caspase activity is modulated by Akt and wherein decreased apoptosis or decreased caspase activity reflects inhibition of acylated homoserine lactone," an express step that is recited in the claims. If anything, they would have suggested to one skilled in art that inhibitors of acylated homoserine lactone should be screened by assessing the Raf-MEK-ERK MAP kinase signaling pathway, not the Akt signaling pathway. Accordingly, Applicants respectfully request withdrawal of the rejection.

Claims 4-5 and 12

The Examiner rejected claims 4-5 and 12 under 35 U.S.C. § 103(a) as allegedly "being unpatentable over Pearson et al. (US 5,591,872, 1997, IDS) in view of Telford et al. (Infection and Immunity, 1998; 36-42) and Maianski et al. (Blood, 2002; 101: 1987-1995, prepublished online as Blood First Edition Paper, October 10, 2002)." Action at pages 4-5.

In addition to reiterating the above points regarding Pearson, the Examiner stated that Telford "teach[es] that the *Pseudomonas aeruginosa* Quorum-Sensing Signal Molecule N-(3-Oxodocecanoyl)-L-homoserine Lactone has immunomodulatory activity and inhibits the production of tumor necrosis factor alpha by lipopolysaccharide-stimulated macrophages (abstract)." Action at page 5. The Examiner also stated that Maianski "teach[es] that the mechanism of apoptosis induction by TNF-$\alpha$ is closely related to the cascade of apoptotic cysteine proteases known as caspases which represent a group of enzymes responsible for

initiation and execution of apoptosis, wherein TNF-α induces apoptosis through the activation of caspases (page 1987, 1st column, 2nd full paragraph and page 1993, 2nd column, 2nd full paragraph)." *Id.*

The Examiner concluded that "[i]t would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to culture a test substance in the presence of N-3-oxodocecanoyl homoserine lactone as taught by Pearson et al. in an animal cell such as a neutrophil and to identify an inhibitor of N-3-oxodocecanoyl homoserine lactone by detecting apoptosis or caspase[] activity in view of the teachings of Telford et al. and Maianski et al." *Id.* For motivation, the Examiner stated that "Telford et al. teaches that 3-O-C12-HSL inhibits TNF-α production which is well known in the art to be involved in apoptosis via the activation of caspases as taught by Maianski et al." *Id.* The Examiner finally contended that:

> [O]ne of ordinary skill in the art would have a reasonable expectation of success that by culturing a test substance in the presence of N-3-oxodocecanoyl homoserine lactone as taught by Pearson et al. in an animal cell such as a neutrophil and to identify an inhibitor of N-3-oxodocecanoyl homoserine lactone by detecting apoptosis or caspase activity in view of the teachings of Telford et al. and Maianski et al., one would achieve an effective method of identifying a suitable inhibitor for the treatment of an immuno-compromised host infected by *P. aeruginosa*, e.g., a person afflicted with cystic fibrosis.

*Id.* at pages 5-6.

Applicants respectfully traverse. Here, the claimed invention is not obvious for at least the reason that Pearson, Telford, and Maianski, whether taken alone or in combination, fail to teach or suggest every element recited in the claims. Again, not one of the documents teaches or suggests "[a] method of screening for a substance that inhibits acylated homoserine lactone, comprising . . . detecting one or more of (a) phosphorylated-Akt, wherein increased

phosphorylation reflects inhibition of acylated homoserine lactone, (b) apoptosis, or (c) caspase activity, wherein the apoptosis or caspase activity is modulated by Akt and wherein decreased apoptosis or decreased caspase activity reflects inhibition of acylated homoserine lactone . . . ."

Applicants direct the Examiner above for the discussion regarding Pearson. Again, the Examiner conceded that Pearson "do[es] not explicitly teach that the method comprising culturing animal cells with the test agent and acylated homoserine lactone and detecting the inhibition of Akt by detecting apoptosis." Action at page 3.

Despite the fact that Telford shows that 3-O-C12-HSL inhibits lipopolysaccharide-induced TNFα production and Maianski shows that TNFα induces apoptosis, the Examiner has not provided any relationship between either lipopolysaccharide-dependent TNFα production or TNFα-induced apoptosis and the Akt signal transduction pathway. Neither Telford nor Maianski suggest that inhibition of the TNFα signal transduction pathway has any bearing on the Akt signal transduction pathway. Moreover, Telford and Maianski do not even mention Akt. Thus, the combination of Pearson, Telford, and Mainski do not teach or suggest anything regarding the Akt signaling pathway. Applicants, therefore, respectfully request the withdrawal of the rejection.

**Conclusion**

In view of the foregoing amendments and remarks, Applicants respectfully request reconsideration and withdrawal of these rejections and timely allowance of the pending claims. Should the Examiner have remaining questions or concerns regarding this application, Applicants request that the Examiner contact the undersigned at (650) 849-6607 to schedule an interview to discuss the application.

Please grant any extensions of time required to enter this response and charge any additional required fees to Deposit Account No. 06-0916.

Respectfully submitted,

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Dated: December 17, 2007

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